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Nodulation and Morphological Responses of Faba Bean (*Vicia faba*) to Water Stress

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Abstract: Water stress responses of faba bean plants vary and are not well understood. Field and greenhouse experiments were conducted to evaluate the responses of seven faba bean genotypes (80S4387, FLIP83-24FB, L82007-11-3-1, FLIP87-26FB, Reina Blanca, FLIP87-70FB and Local Check) to soil moisture conditions. Data were collected on plant root and shoot dry weight, leaf area, plant height, root volume, total root length, nodule dry weight and nodule number of seven faba bean genotypes. In the field, a split block design with three replications was used to evaluate two water levels (irrigation and rainfed) as main plot treatment and seven faba bean genotypes as sub-plot treatment. Whereas the greenhouse trial had a factorial combinations of three water levels (30, 60 and 90% of soil field capacity) and seven genotypes. Water deficit reduced the growth of plant shoot and root morphological traits. Higher reduction in average nodule dry weight per plant was occurred under rainfed (water deficit) conditions as compared to the irrigated plants. The extent of adaptation of genotypes to water stress with respect to shoot and root morphological parameters was quite variable. Accordingly, Reina Blanca, followed by L82007-11-3-1 and FLIP87-26FB had preformed better under water stress conditions and can be used in future breeding programs.

Key words: Faba bean · Nodulation · *Vicia faba* · Water stress

INTRODUCTION

Agriculture in Eastern Mediterranean suffers from inadequate rainfall and erratic rain distributions. Likewise, moisture shortage is the major determinants of crop production in arid and semiarid climates. Autumn and winter sown crops under Mediterranean conditions are likely to experience intermittent and terminal drought stress during vegetative and reproductive periods [1] and root growth is often confined to the upper soil layers [2].

Drought and heat stress can devastate a faba bean crop [3]. Summer seasons of arid and semiarid regions are characterized by hot, dry and high light intensity which raise the demand on atmospheric vapor pressure for both plants and soils, resulting in significant agricultural losses. The limited capacity of the soils to infiltrate and store the scanty and erratic rainfall in Mediterranean countries [4] plays a significant role in making the situations worse.

Root growth is often a good indicator of plant's ability to withstand water deficit and waterlogged conditions. Deep and extensive root systems play a major role in drought tolerance of the plant [5-7]. Leaf area is an important variable for most physiological and agronomic studies [8, 9]. The water stress is the major factor affecting the leaves expansion rate and the total leaf area of the plants [7, 10, 11].

Although free-living microorganisms contribute to the pool of fixed nitrogen in agricultural systems, the legume-Rhizobium symbiosis is the most important one. However, water stress had major deleterious effect on nodulation and therefore the symbiotic nitrogen [12].

Water stress causes various root and shoot morphological changes, however, many of which are not well characterized and understood especially in cool season food legumes such as faba bean [2, 13, 14]. Therefore, this study was evaluated the responses of the root, shoot and nodulation of seven-faba bean genotypes to water stress under greenhouse and field conditions.

MATERIALS AND METHODS

Experiments were conducted during the 2001/2002 growing season in the field and greenhouse at Jordan University of Science and Technology (JUST) campus Information as to the names, origin and pedigree of the

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Table 1: Names, pedigree and origin of faba bean genotypes used in greenhouse and field experiments

Genotype	Pedigree	Country of origin
80S4387	X77TA31 E 88159X Ì88160	ICARDA[z] (Syria)
FLIP83-24FB	Selection 81S36522	ICARDA (Syria)
L82007-11-3-1	EILB 1817 X ¥76TA56246	ICARDA (Syria)
FLIP87-26FB	S82148 E 50088X ¥ 79S79180	ICARDA (Syria)
Reina Blanca	ILB 1270	United Kingdom
FLIP87-70FB	S 82148 E 50088X ¥S79180	ICARDA (Syria)
Local Check	Unknown	Jordan

[z]ICARDA= International Center for Agricultural Research in the Dry Areas

genotypes is presented in Table 1. The total amount rainfall received and irrigation water applied during the growing season was 167 and 268 mm, respectively (Fig. 1). Moreover, the mean maximum and minimum temperature and the relative humidity of the growing season are depicted in Fig. 2.

Greenhouse Experiment: Five seeds of each genotype were sown into a 5-L plastic pot that was filled with 4.85 kg of 2:1 (V:V) clay to sand soil mixes on the 5th of July 2001. Seedlings were thinned to three plants per pot one week after emergence. Soil moisture was measured using gravimetric method to set moisture contents of 30, 60 and 90% of the soil field capacity. Pot moisture contents were monitored every 24 hours by weighing; then after, pots were irrigated to restore the weight at those of field capacity levels throughout the course of the experiment. The experiment was conducted in a 3×7 factorial arrangement of treatment combination in a randomized complete block design (RCBD). The 21 treatments of genotype-moisture content combinations were replicated three times. The 63 pots were arranged on a bench in the greenhouse side by side in triple rows under natural light conditions. Diammonium phosphate (DAP) (18-46-0) at a rate of 30kg/ha was applied as starter fertilizer at time of planting. This experiment was terminated at the end of the sixth week from the inception of treatment application.

Field Experiment: In a separate field experiment, seeds from the genotypes described above were hand sown on the 15th of January 2001 in four rows per plot. Plots were 3 m long and 1.6 m wide. Inter and intra row spacing was 0.40 and 0.10 m, respectively. A population density of 25 plants per square meter was reached in all plots. Moreover, two-meter and three-meter borders were left unplanted between plots and between replicates, respectively. Weed control was performed manually twice throughout the course of the experiment. Diammonium phosphate (DAP) (18-46-0) was applied at time of planting. Drip irrigation system was used to supply irrigation water. A Split block design with three replications was utilized. Water stress levels (irrigation vs. rainfed) were assigned to main plots while genotypes were randomly assigned to sub-plots.

Data Collection

Leaf Area: Average of three plants leaf area was determined upon terminating the greenhouse experiment and at the late pod filling stage of the field experiment by using a LI-COR 3100 leaf area meter (LI-COR, Inc., Lincoln, NE[2]).

Plant Height : Plant height was also measured on 5 randomly selected plants of field experiment at late pod filling stages and on three plants per pot upon termination of greenhouse study.

Root Parameter Measurement: Pots in which shoot parts have been removed at crown point for shoot dry weight determination, were submerged in plastic container filled with water for 24 hrs. Then roots were isolated. After it was thoroughly washed with tap water and rinsed with distilled water, the roots were kept in graduated cylinders containing distilled water. Root volumes were determined by the volume displacement methods. Roots were then stored in plastic bags and frozen until total root length was measured. Before cutting the whole root into 1-2 cm root segments for the determination of total root length using Newman [15] method, it was blotted and weighed for fresh weight (W1). Whole root segments were put in a big beaker and well stirred with 1L of distilled water. Two gram root segments samples were randomly drawn from the beaker and spread on a clean paper without allowing any overlap to facilitate easy counting of root segments. After counting the root sample segments, total root length (RL) per plant was calculated as:

RL = (Root fresh weight × 1.5 × segment number)/ Mean weight of samples segment

The oven-dry weight of the plant root was determined by drying sample root segments used for root length determination and the original root at 75°C for 24 h. During the early flowering stage of the field experiment, four random plants from each plot were carefully pulled out with their roots intact by digging the soil to approx. 40 cm. Roots were washed gently with tap water and rinsed to remove soil particles to collect clean nodules. Nodules were counted and dry weights were determined after drying at 75°C for 24 hrs.

Statistical Analysis: Statistical analysis was performed using MSTATC (Michigan State University [16] as outlined for a two factor randomized complete design (greenhouse) or for a Split block design (field experiment). Means were separated according to fisher Least Significant Difference (LSD) at 0.05 level of significancy.

RESULTS AND DISCUSSION

In the greenhouse experiment, plants exposed to severe water deficit levels (30% field capacity) did not survive beyond the third week after inception of treatments application, therefore it was not included in the analysis. Significant water level × genotype interactions were noted for shoot dry weight, root volume, root dry weight, nodule dry weight as well as leaf area and plant height of the field experiment (Table 2 and Fig. 3, 6, 7). On the other hand, variation in total root length, nodule number early leaf area and plant height of greenhouse studies (Table 3) were significantly differed only for main effects (genotypes and water levels).

Effect of Water Stress on Shoot Parameters

Shoot Dry Weight: Shoot dry weight of faba bean respond significantly to water level × genotype interactions (Fig. 3). The highest shoot dry weight was obtained in Reina Blanca genotype. While drought has generally affected shoot dry weight, it can be deduced that some faba bean genotypes L82007-11-3-1 and Reina Blanca had tended to have higher shoot dry weights. The ability of these genotypes to accumulate higher dry matter under water deficit conditions indicates a high capacity to adaptate to water stress through better exploiting the limited water quantity. Similar results reported by Neal and McVetty [17] and Khan [18].

The considerable reductions that were observed in shoot dry weights under water deficit conditions could be attributed to a loss in turgidity of faba bean plants and hence the cells are unable to multiply, divide and expand or absorb nutrients for the normal growth and development. More increment in shoot dry weight under non-water deficit conditions may be attributed to enhanced leaf area expansion (Table 2) and the consequent high absorption of photosynthetically active radiation (PAR) early in the season compared to plants grown under water deficit conditions.

Leaf Area: Significant variations in leaf area responses to water levels and genotypes were observed in the both field and greenhouse trials (Table 2 and Fig. 3). However, variations in response to water level × genotype interaction were insignificant in early evaluation of greenhouse study (Fig. 3). More inhibitions in average leaf area expansion was occurred under water deficit conditions in comparison with non-water-stress condition. The present result is in conformity with that of Farah [10] and Khan [18] on different beans (*Vicia faba* L.) genotypes. He reported an early reduction of leaf area expansion under water deficit conditions. Such reductions might be accounted for adverse of water deficit on number, volume and arrangement of the cells [10]. It is worth noting that in the greenhouse study, Reina Blanca followed by L82007-11-3-1 attained considerably highest leaf area expansion (Table 2), which indicates high adaptability to drought conditions. In the field experiment, L82007-11-3-1 had resulted in highest leaf expansion area under irrigated conditions, however, it was not greatly varied from the values obtained in three of the genotypes viz. Reina Blanca, 80S4387 and FLIP87-26FB. On the other hand, the local genotype had produced the lowest leaf area, but the variation was statistically insignificant from that of FLIP87-70FB. Under rainfed conditions, genotypes showed similar leaf area expansion (Fig. 4).

Plant Height: Plant height under greenhouse condition (Table 2) and at late pod filling stage of field study (Fig. 5) showed significant responses to water stress. However, plants did not respond to water levels × genotype and genotype effect under field experiment. Under field study, plants of FLIP87 -26FB and FLIP83-24FB (under irrigated conditions) and Reina Blanca and FLIP83-24FB (under rainfed conditions) was the tallest (Fig. 5).

Water deficit reduced plant height. Hebblethwaite [11] reported the deleterious effects of water deficit on height and stem growth of faba bean. The decline in plant height under water stress conditions could be attributed to the reduction on number, volume and arrangement of the cells [10]. Apart from direct role of water in promoting plant height; water availability may increase the duration of growing season. The decline in leaf area expansion (Table 2 and Fig. 5) and the subsequent limitation of assimilate supply may have been attributed to the early decline of plant height under water deficit conditions.

Effects of Water Stress on Root Parameters

Root Dry Weight: Reina Blanca and L82007-11-3-1 produced the highest yield under non-stress and water stress conditions, respectively. In the other extreme, the local genotype had produced the lowest root dry weight under both water stress levels (Fig. 6). Moreover, considerable differences in root dry weight were also noted in response to water stress levels and genotypic

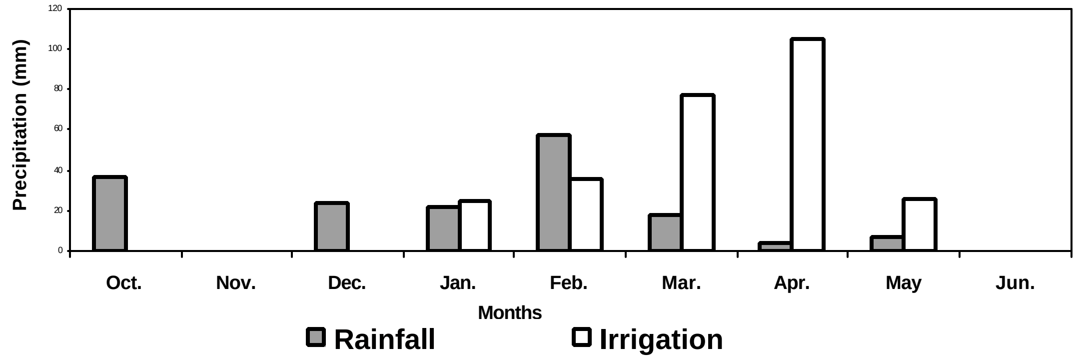

Fig. 1: Monthly rainfall received and supplementary irrigation applied during the 2000/2001 growing season at JUST (rainfall data obtained from Ramtha Regional Agricultural Service Center)

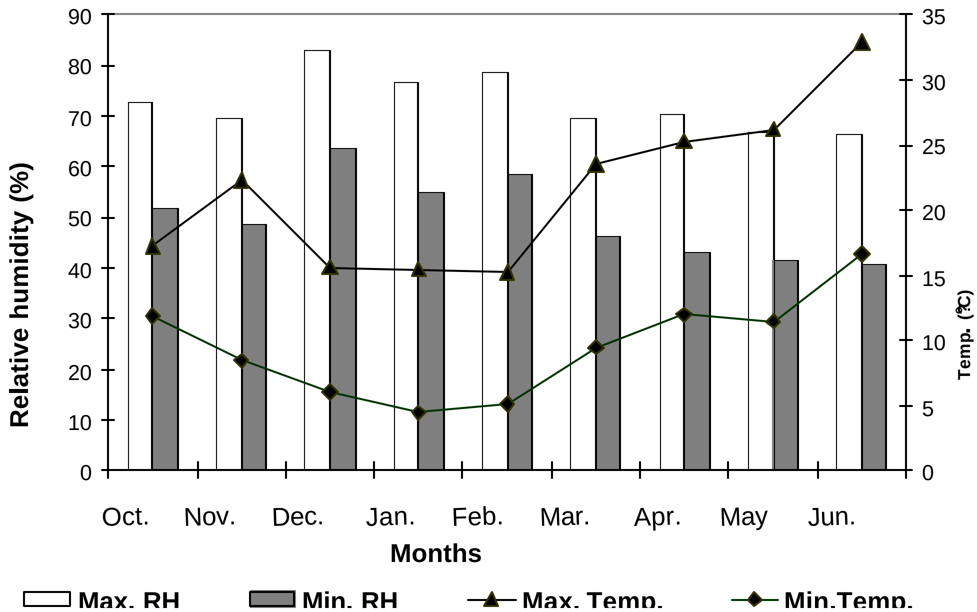

Fig. 2: Mean maximum and minimum relative humidity (bars) and temperatures (lines) during the 2000/2001 growing season at JUST (data obtained from Ramtha Regional Agricultural Service Center).

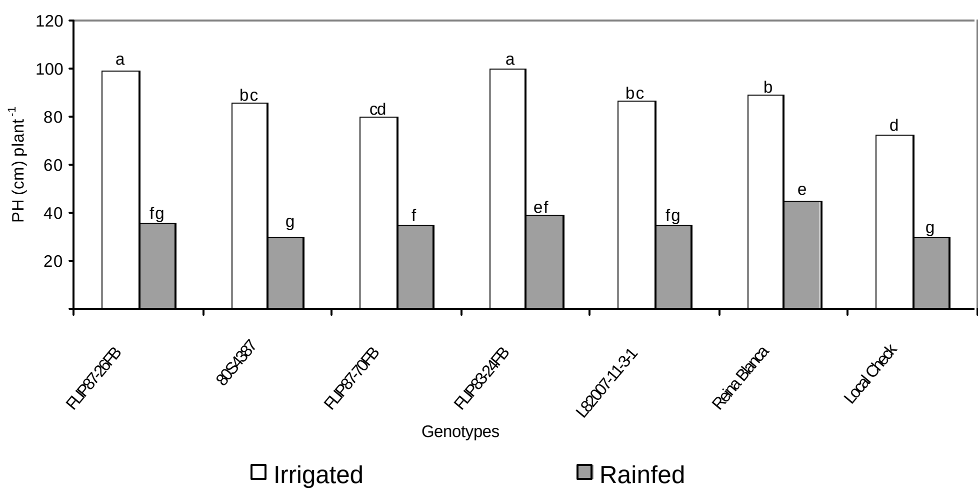

Fig. 3: Effect of water stress on shoot dry weight of seven faba bean genotypes grown under greenhouse condition (Bars capped with same letter(s) are not significantly different at (P≤0.05) according to LSD test

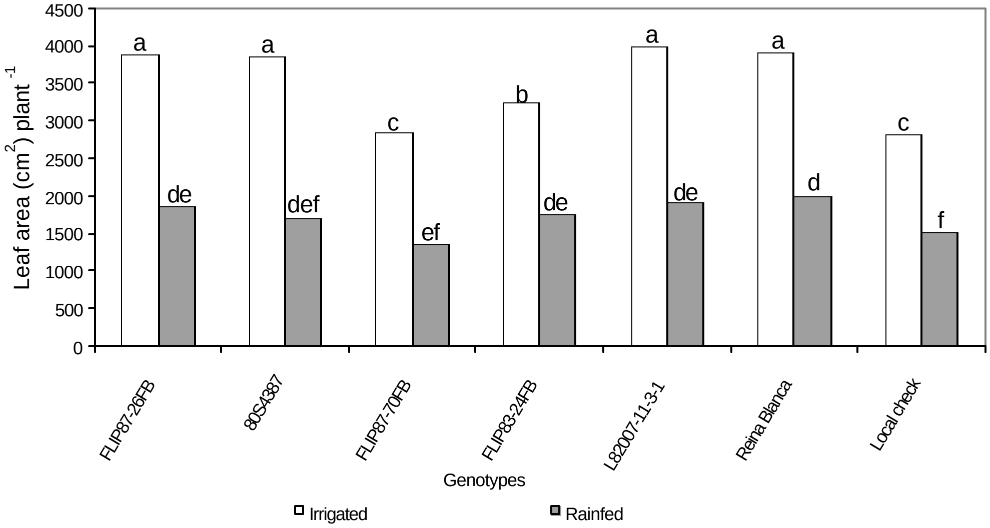

Fig. 4: Impact of water stress on leaf areas of seven faba bean genotypes grown under field conditions during the 2001 growing season at JUST (Bars capped with same letter are not significantly different at (P≤0.01) according to LSD test)

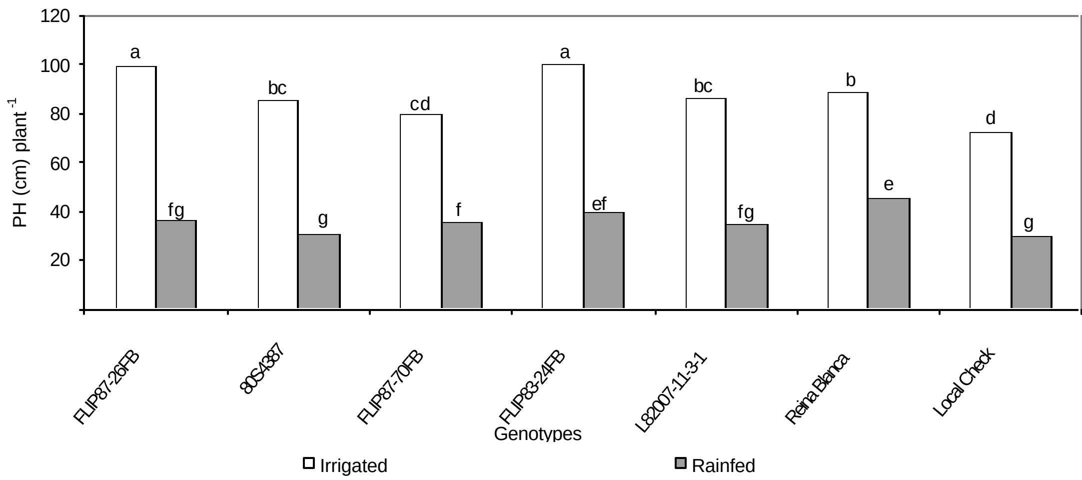

Fig. 5: Effect of water stress on plant height (PH) of seven faba bean genotypes grown under field conditions during the 2001 growing season at JUST (Bars capped with same letter (s) are not significantly different at (P≤0.01) according to LSD test)

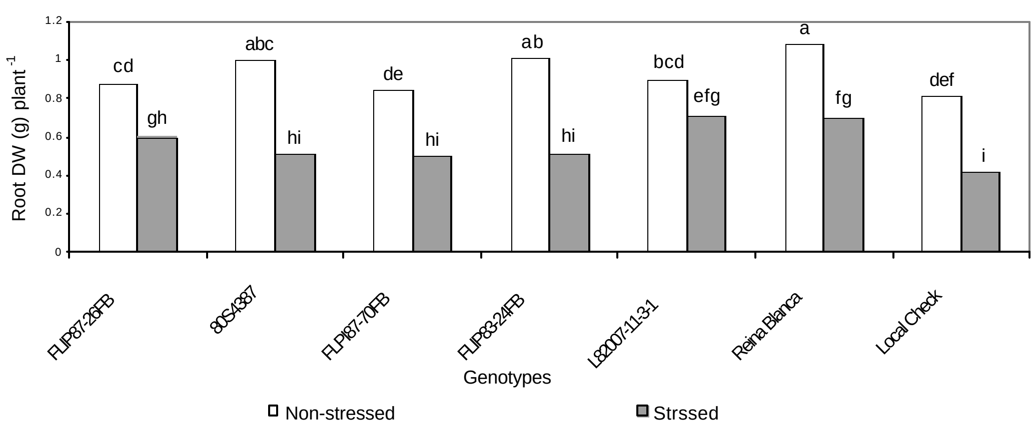

Fig. 6: Influence of water stress on root dry weight (DW) of seven faba bean genotypes grown under greenhouse conditions during 2001 growing season at JUST (Bars capped with same letter(s) are not significantly different at (P≤0.01) according to LSD test)

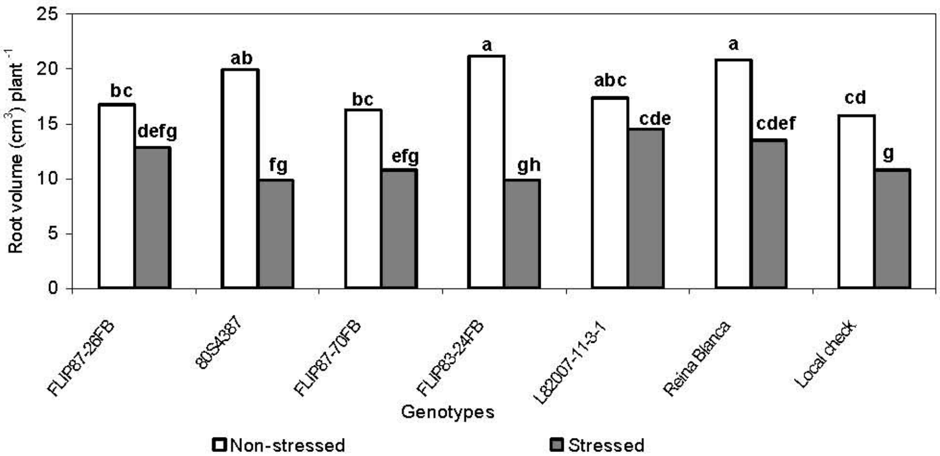

Fig. 7: Effect of water stress on root volume of seven faba bean genotypes grown under greenhouse conditions during the 2001growing season at JUST (Bars capped with same letter(s) are not significantly different at (P<0.01) according to LSD test)

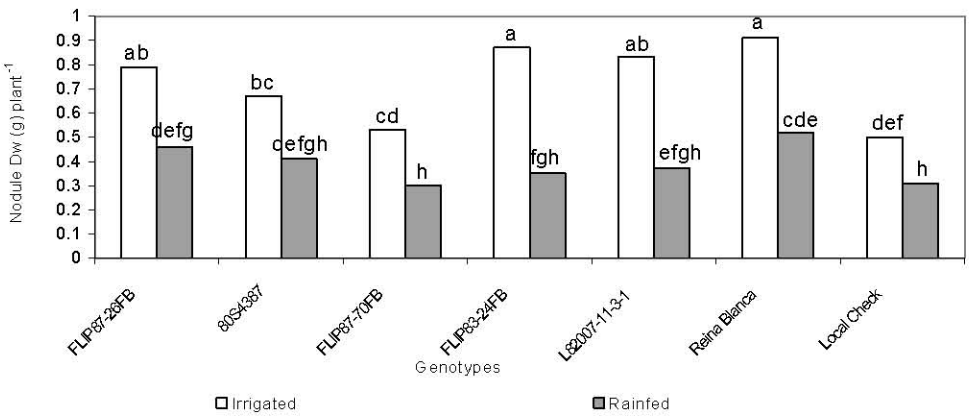

Fig. 8: Effect of water stress on nodule dry weight (DW) of seven faba bean genotypes grown under field conditions during the 2001 growing season at JUST (Bars capped with same letter(s) are not significantly different at (P<0.01) according to LSD test)

variations. Lower average root dry matter yield per plant was obtained from plants grown under water deficit conditions. The decrease in root dry weight under water deficit conditions may be attributed to the considerable decline in leaf area expansion and subsequent limitation of assimilates for normal root growth. Water stress inhibits nutrient uptake and decreases nitrogen fixation [19] due to the dramatic reduction in nodule number (Table 3) and nodule dry weight (Fig. 8), which might reduce root dry weight. In an earlier investigation undertaken on beans and sorghum genotypes, similar results were reported which indicated the decline in root dry matter yield as water stress increased [2]. Moreover under in vitro conditions, a very recent report has indicated the inhibitory effects of induced water deficit on root dry matter yield of "Nabali" olive seedlings [20].

Root volume: Mean values of root volume per plant are presented in Table 1. Significant water level × genotype interactions was noted in faba bean root volume (Fig. 7). Plants of FLIP83-24FB grew at 90% soil F.C. and L82007-11-3-1 grew at 60% soil F.C. produced the highest root volume, whereas the local genotype was inferior in root volume under both water levels (Fig. 7). Similarly, AL-Karaki *et al.* [2] have also reported the existence of significant water level × phosphorous and phosphorus × cultivars interactions for root volume and total root length on beans.

Table 2: Leaf Area (LA) and Plant Height (PH) of seven faba bean genotypes grown at two water levels under greenhouse conditions

Genotypes	Non-stress LA (cm^2) plant $^{-1}$	Water stress LA (cm^2) plant $^{-1}$	Mean LA (cm^2) plant $^{-1}$	Non-stress PH (cm) Plant $^{-1}$	Water-stress PH (cm) Plant $^{-1}$	Mean PH (cm) Plant $^{-1}$
FLIP87-26FB	956.54	524.22	740.38	53.11	27.24	40.17
80S4387	967.10	435.13	710.11	48.32	28.47	38.40
FLIP87-70FB	898.10	466.58	682.34	44.67	25.55	35.12
FLIP83-24FB	875.74	449.30	662.52	51.20	26.89	39.05
L82007-11-3-1	1039.90	542.10	791.00	50.33	29.45	39.90
Reina Blanca	1175.70	584.88	880.29	47.69	29.72	38.78
Local Check	824.79	376.71	600.75	41.64	26.48	34.24
Mean	962.55	482.70		48.56	27.74	
LSD 5%			NS			NS

Probability of significance Source	df	Leaf area	Plant height
WS levels (WS)	1	<0.01	<0.01
Genotype (GT)	6	>0.05	>0.05
WS × GT	6	>0.05	>0.05

Table 3: Total root length (RL) and nodule numbers per plant of seven faba bean genotypes grown at two water levels under greenhouse and field conditions, respectively

Genotypes	Non-stressed RL (m) Pl.$^{-1}$	Stressed RL (m) Pl.$^{-1}$	Mean RL (m) Pl.$^{-1}$	Irrigated Nodules pl $^{-1}$	Rainfed Nodules pl $^{-1}$	Mean Nodules pl $^{-1}$
FLIP87-26FB	5.55	3.29	4.42	153.89	93.89	123.89
80S4387	6.33	3.42	4.88	120.11	83.00	101.56
FLIP87-70FB	6.46	2.93	4.70	128.55	74.00	101.28
FLIP83-24FB	6.75	2.63	4.69	123.83	70.11	96.97
L82007-11-3-1	7.18	3.08	5.13	130.33	74.89	102.61
Reina Blanca	7.83	4.16	6.00	152.26	85.19	118.73
Local Check	2.63	1.59	2.11	127.39	73.33	100.36
Mean	6.10	3.01		133.77	79.20	
LSD 1%			1.41			15.92

Probabilities of significance Source	Df	RL	Nodule number
Water level (WS)	1	<0.01	<0.01
Genotypes (GT)	6	<0.01	0.028
WS×GT	6	>0.05	>0.05

A considerable reduction in average root volume was noted under water deficit than non-water stress conditions. The reductions in root volume of the present study do agree with the report on sorghum cultivars, which indicated the decline of root volume with elevated water deficit [2]. In the case of genotypes response over the two growth conditions, Reina Blanca had resulted in a significantly highest average root volume per plant though it was statistically the same with the value occurred in four of the genotypes studied namely L82007-11-3-1, 80S4378, FLIP87-26FB and FLIP83-24FB.

Total Root Length: Total root length was significantly affected in response to water levels and genotypic variations. Nevertheless, the impacts of water levels × genotype interactions were insignificant (Table 3). Higher average total root length per plant was occurred under non-water stress conditions as compared to water stress grown plants. The present results are in agreement with reports on beans [2]. In this particular study the authors have found a dramatic reduction of total root length under prolonged water deprivation regardless of the phosphorus levels applied, which apparently believed to enhance the plant adaptation to water deficit through promotion of early root growth [21, 22]. The observation recorded in the present study also agrees with the very recent report on four groundnut cultivars [23].

Among genotypes studied, Reina Blanca had resulted in a more pronounced average total root length over the two water stress level, however, the variation was insignificant from values attained by most of the genotypes except, FLIP87-26FB and the local check. The shortest root length was obtained from the local genotype (Table 3). This fact indicates that some genotypes do have the ability to cope better with water deficit or postpone water deficiency effects through exploring deeper sub soil layers through extensive root systems. The relative increase in total root length of some of the genotypes (such as Reina Blanca, 80S4387, FLIP87-26FB and L82007-11-3-1) under water deficit conditions may be a morphological adaptation to improved water uptake and postpone dehydration [24]. Evidences that support the existence of genetic variability in root length among genotypes have been provided in an investigation undertaken in cool season food legumes [25] as well as in 12 faba bean genotypes [26].

Effects of Water Stress on Nodulation: There were significant differences in nodule numbers per plant in response to water stress levels and genotypic differences. Consequently, water deficit had adversely affected the average nodule number (79.33), whereas a higher nodule numbers (133.77) were obtained from irrigated plants (Table 3). Zablotowicz *et al.* [27] reported similar water stress effects on root nodule number of common bean (*Phaseolus vulgaris* L) and faba bean (*Vicia faba* L.) plants.

Under greenhouse and field conditions, FLIP87-26FB and Reina Blanca plants had been produced the highest nodules number (Table 3). However, the nodule numbers produces by other genotypes were low. Islam [28] reported potentials variation in nodule production due to presence of genetic variation.

Significant water stress level × genotype interactions was found in nodule dry weight of faba bean plants. Under irrigated conditions, Reina Banca had ranked first (0.91 g/plant); however, this was not statistically differ from the nodule dry weight reported for FLIP87-26FB, FLIP83-24FB and L82007-11-3-1. Reina Blanca followed by FLIP87-26FB produced more nodule dry weight under rainfed conditions; whereas the local genotype, FlIP87-70FB and FLIP8324FB were inferior in nodule dry weight per plant (Fig. 8).

In addition, significant variations in nodule dry weight were detected in response to water stress and genotypic variations. Higher reduction in average nodule dry weight per plant was occurred under rainfed (water deficit) conditions as compared to the irrigated plants. The current results are in agreement with the findings of Day and Legg [19] and Sprent [29] on another faba bean genotypes. The reduction of nodule dry weight under water deficit conditions may be due to the inhibition of infection process and/or through its adverse effect on population of Rhizobia in the rhizosphere [12].

CONCLUSION

This study has shown genotypic diversity for drought associated morphological traits and nodulations. Based on the results attained, it seems that shoot and root morphological traits are useful in describing the imposed water stress due to their sensitivity to plant water status. Reina Blanca followed by L82007-11-3-1 and FLIP87-26FB had resulted in enhanced shoot and root morphological traits even if genotype × environment interaction exists for some of the traits.

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